

Testing and comparison of freshwater leech repellents

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Leeches are an important part of many freshwater ecosystems, but they can be a nuisance and a health hazard for humans who want to enjoy a swim. To keep them off the legs of swimmers, we set out to find effective repellents that were also relatively safe for the environment. We applied five treatments (negative control, 5% distilled white vinegar, 40% DEET insect repellent, 30% Lemon Eucalyptus oil insect repellent, chewing tobacco and water mixture) to bait and counted how many freshwater ribbon leeches (*Erpobdella obscura*) attached to the bait for each treatment. We hypothesized that all treatments would be more effective than the control at repelling leeches, and specifically predicted that tobacco would be most effective. We found that vinegar and the control were not effective and the other three treatments were effective; no relationship was found between a repellent's acidity and its effectiveness. Going forward in the quest to protect swimmers, it would be beneficial to test the three successful repellents on bloodsucking leeches as we only had access to a jawless species that dines on invertebrates.

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Introduction

Leeches are annelid worms in the subclass *Hirudinea* that occur across the globe, terrestrially and aquatically. Many aquatic species are predatory and eat only small invertebrates, even though leeches are infamously known as parasites that feed on vertebrate blood. Apart from preying on other invertebrates and parasitizing vertebrates, leeches are also an important prey species for many animals in freshwater ecosystems. Locally, their predators include birds, starry-nosed moles, turtles, trout and many other fish.

People who are not ecologists are unlikely to view leeches in a favorable light. When a hematophagic leech bites a human, it secretes an anticoagulant into the wound to keep blood from clotting, meaning the bite may bleed long after the leech is gone. When a person attempts to remove a leech incorrectly, such as with salt or flame, the leech regurgitates its stomach contents into the wound before detaching, potentially causing infection (Butler 2012). While leeches are not generally known as keystone species, removing all the leeches in an ecosystem would decrease biodiversity and therefore have a negative impact on the ecosystem (Rapport et al., 1998). Because killing leeches en masse is a poor option, repelling them is the next best choice for humans who do not desire to be parasitized.

Multiple scientific studies have focused on deterring hematophagic terrestrial leeches. Processed oil of the lemon eucalyptus plant (*Corymbia citriodora*) was effective on Malaysian land leeches when combined with protective clothing (Kirton 2005). DEET, the commonly used insect repellent, was also effective against land leeches (Gouck 1966). Terrestrial leeches are not an issue in Michigan, but attracting freshwater leeches while swimming is relatively common. Our goal with this study was to evaluate the efficacy of probable freshwater leech repellents that could be applied directly to the skin, remain effective in water, and would not negatively impact freshwater ecosystems.

We hypothesized that all four experimental treatments (lemon eucalyptus oil insect repellent, DEET insect repellent, water soaked in chewing tobacco, white vinegar), would work significantly more effectively than the negative control. We predicted that tobacco (*Nicotiana tabacum*) would work most effectively because a study in Iran showed that nicotine was toxic to aquatic leeches (Bahmani et al., 2012). We also compared the acidity of each treatment to its effectiveness to see if there was a relationship.

Materials and Methods

We acquired one hundred twenty freshwater ribbon leeches (*Erpobdella obscura*) from a fish bait supplier in Alanson, Michigan in July 2016. The leeches were caught wild in the Crooked River, a short river that flows northeast from Crooked Lake at (45°25'58"N, 84°47'19"W) into Burt Lake at (45°28'14"N, 84°43'38"W).

To fill the housing and experimental tanks, we used water exclusively from the southern shore of Douglas Lake (45°34'N, 84°42'W) near Pellston, Michigan. We separated individuals equally into twenty experimental tanks (six leeches per 38 L tank filled with 3 L water at 25°C) the night before testing, giving them enough time to resettle. We subjected every tank to a single treatment, eliminating the variable of multiple exposures to different treatments. We performed four replicates for each of the five treatments, allowing us to account for variability among individuals.

Our five treatments included: a negative control; 30% lemon eucalyptus oil insect repellent; 40% DEET insect repellent; water mixed 1:1 by volume with Copenhagen Original moist snuff chewing tobacco; and 5% distilled white vinegar. We chose white vinegar (pH 2.7) because while highly acidic lakes (≤ 4.9) in Ontario do not contain leeches, the short term negative effects of acidity have not yet been studied on leeches (Bendell and McNicol 1991). DEET impacts insects' odor receptors, confusing them, but it is unclear if freshwater leeches rely heavily on olfaction to find food (Pellegrino, et al. 2011). As previously mentioned, DEET and lemon eucalyptus oil extract have already been found effective at repelling terrestrial leeches, and tobacco has been found to negatively impact aquatic leeches.

We applied each treatment to our bait, an approximately 100 g piece of raw beef liver brought to room temperature, in a way that mimicked application to human skin. We sprayed each insect repellent treatment onto the bait and dipped the bait in the tobacco-water and vinegar treatments. We suspended treated bait under the water level in each tank and waited.

Our dependent variable was the percentage of leeches that attached to the bait in a 30 minute period. We measured the pH of each treatment before application and recorded attachments under three subdivisions of time (0-10 min, 11-20 min, 21-30 min). We used ANOVA to compare percentage of leech attachments within 30 minutes among treatments, and linear regression to determine if there was a relationship between percentage of attachments and treatment pH.

Results

pH.—No statistically significant relationship existed between the effectiveness of a treatment and the treatment's pH (Figure 7). Vinegar was the most acidic treatment, yet it was the experimental treatment that worked worst (Figure 1).

Leech attachments among treatments.—Tobacco, lemon eucalyptus oil insect repellent, and DEET insect repellent all showed significantly fewer leech attachments than the control, while the percentage of attachments for the vinegar treatment was not significantly different than the control (ANOVA, $F = 43.671$, $df = 4$, $p < 0.000$; Figure 1).

Control treatment.—The majority of control treatment leeches attached to the bait within the first 10 minutes (Figure 2); on average, 91.5% of the leeches attached to the bait in each full length trial (Table 1).

Vinegar treatment.—Vinegar demonstrated more continuous attachment over time than the control (Figure 3); on average, 66.6% of the leeches attached to the bait in each vinegar treatment tank (Table 1).

Tobacco treatment.—Tobacco had a single leech attachment occurring within the last 10 minutes in one of the four trials (Figure 4).

Lemon eucalyptus oil treatment.—Lemon eucalyptus oil insect repellent had zero attachments in 30 minutes (Figure 5).

DEET insect repellent treatment.—DEET insect repellent had a single attachment in the 11-20 min division, and in another tank, two attachments in the 21-30 min division (Figure 6); on average, 12.5% of the leeches attached to the bait in each DEET treatment tank (Table 1).

Discussion

Our hypothesis that all treatments would be more effective than the control was only 75% accurate because vinegar did not work statistically better than the control. No relationship was found between acidity and treatment effectiveness, which we found surprising. We rejected the idea that short-term acidity repelled leeches in the same way that long-term acidity prevented leech species from surviving in lakes.

Although DEET and lemon eucalyptus oil insect repellents were only tested on terrestrial hematophagic leeches in previous studies we found, their additional success on predatory freshwater leeches suggested they may work on both kinds of leeches via the same mechanism. A potential mechanism for lemon eucalyptus oil was not proposed, but DEET affects insects, and possibly leeches, via their odor receptors. The nicotine in tobacco is an effective insecticide due to the muscle weakness it causes by interacting with insects' acetylcholine receptors (Bahmani et al., 2012); it is possible that our tobacco treatment affected the leeches in a similar manner.

We did not consider the water solubility of each treatment prior to testing, but believe this factor may have been important. The vinegar was already diluted with water before application and may have further dissipated in the tank water as testing progressed, resulting in its poor performance. The DEET spray was not waterproof and may have worn off throughout the 30 minute testing period, leading to the slight upwards trend in attachments as time progressed. The lemon eucalyptus oil insect repellent, with "oil" in the name perhaps implying hydrophobicity, may have been less soluble than the other treatments and adhered to the bait best. Chewing tobacco bits remained visibly adhered to the bait throughout the full testing period.

Unfortunately we were unable to obtain local bloodsucking leeches for our experiment. We had to assume *E. obscura*, a freshwater species that only eats invertebrates, well-enough represented the way hematophagic freshwater leeches hunt and are deterred for our study to be considered applicable. We also assumed the beef liver bait was a suitable substitute for human flesh.

Keeping the leeches healthy proved difficult, which may have impacted our results; half of our original leeches died and were replaced the day before trials were run, and one mortality occurred in a control tank while we ran the trials. Many individuals appeared lethargic, lumpy, and had a gelatinous white cloud on their posterior suckers. Future studies would benefit from using proper leech housing techniques and hematophagic leech species, as well as quantifying each repellent's water solubility.

Literature Cited

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Tables and Figures

Table 1. Descriptives of leech attachments in percentages.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
Control	4	.9150	.09815	.04907	.7588	1.0712	.83	1.00
Vinegar	4	.6658	.19140	.09570	.3612	.9703	.50	.83
DEET	4	.1250	.15946	.07973	-.1287	.3787	.00	.33
Tobacco	4	.0418	.08350	.04175	-.0911	.1746	.00	.17
LemEuc	4	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	20	.3495	.39632	.08862	.1640	.5350	.00	1.00

Figure 1. Bar graph with 95% confidence interval bars showing average percentage of leeches that attached to the bait for each treatment. Dots denote pH of each treatment prior to application. There is no statistically significant difference among treatments labeled with the same letter (A or B) (ANOVA, $F = 43.671$, $df = 4$, $p < 0.000$).

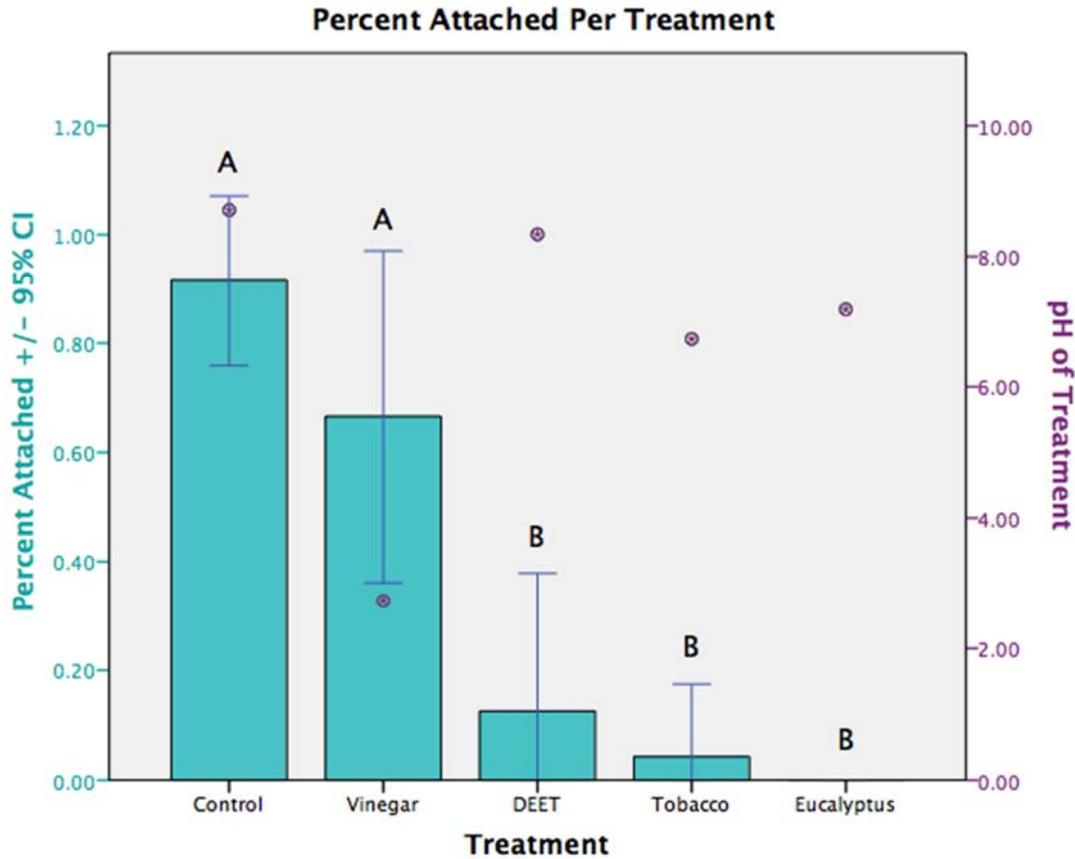


Figure 2. Control treatment pie chart showing average percentage of leeches that attached to the bait by time division, as well as percentage of leeches that did not attach at all.

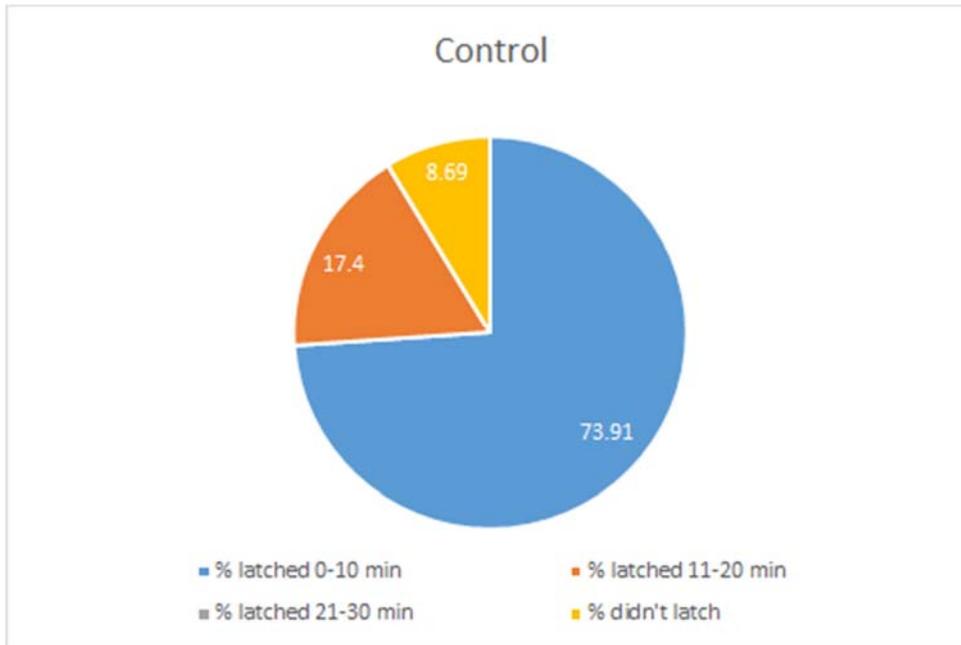


Figure 3. Vinegar treatment pie chart showing average percentage of leeches that attached to the bait by time division, as well as percentage of leeches that did not attach at all.

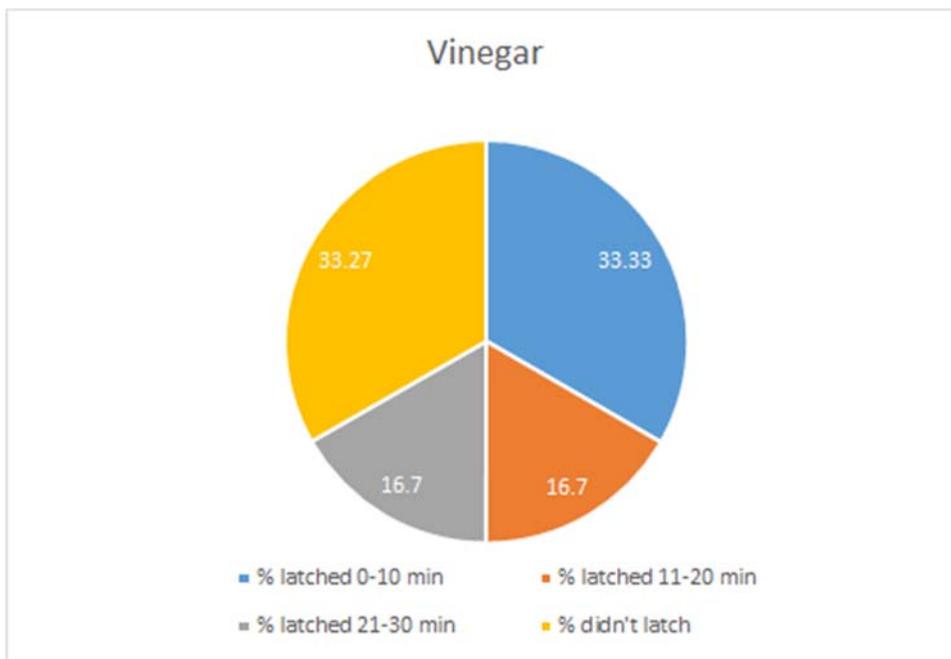


Figure 4. Tobacco treatment pie chart showing average percentage of leeches that attached to the bait by time division, as well as percentage of leeches that did not attach at all.

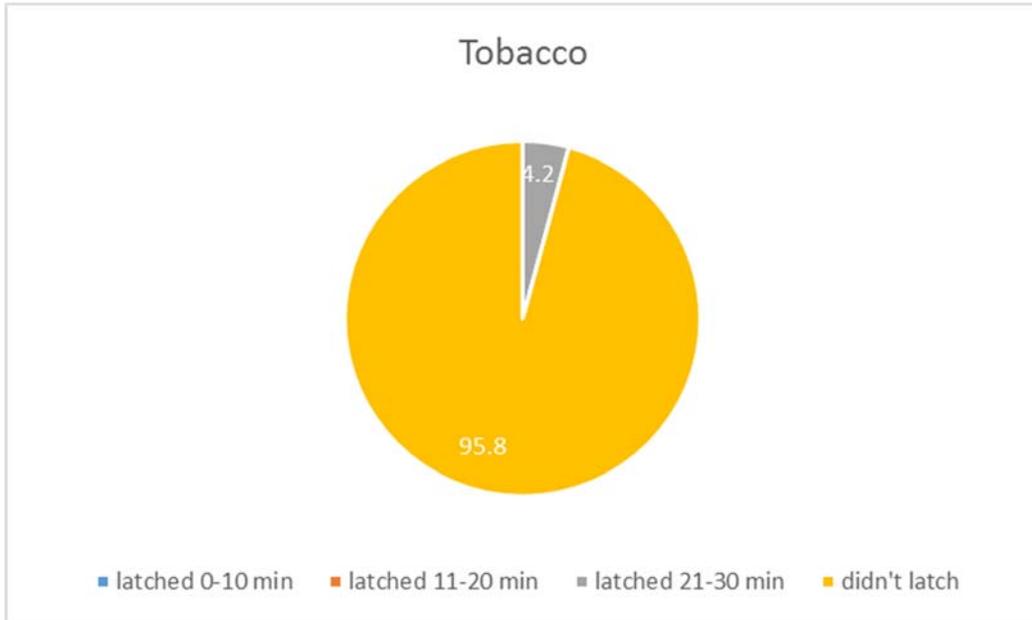


Figure 5. Lemon eucalyptus oil insect repellent treatment pie chart showing average percentage of leeches that attached to the bait by time division, as well as percentage of leeches that did not attach at all.

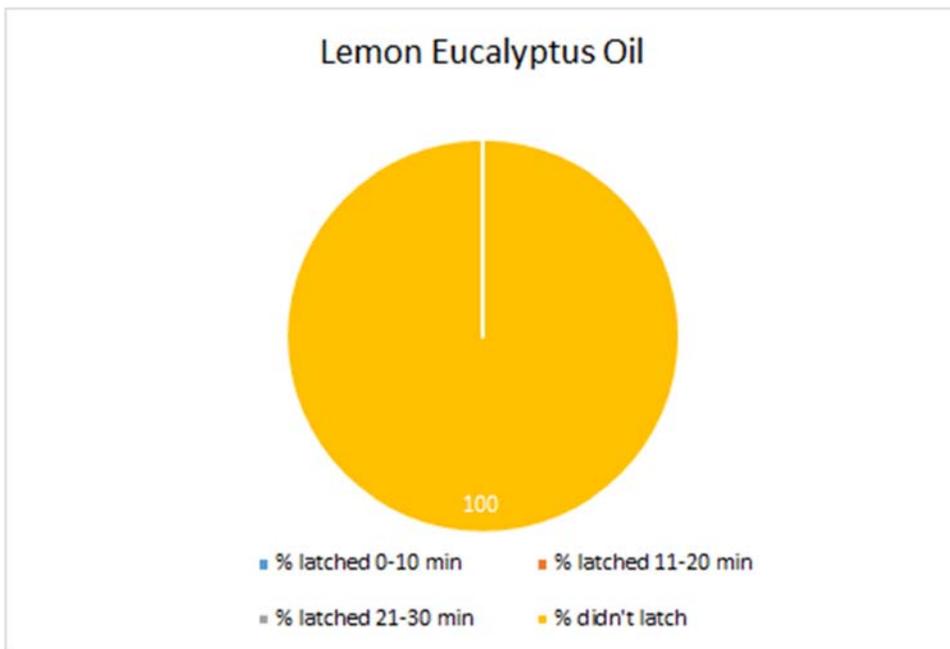


Figure 6. DEET insect repellent treatment pie chart showing average percentage of leeches that attached to the bait by time division, as well as percentage of leeches that did not attach at all.

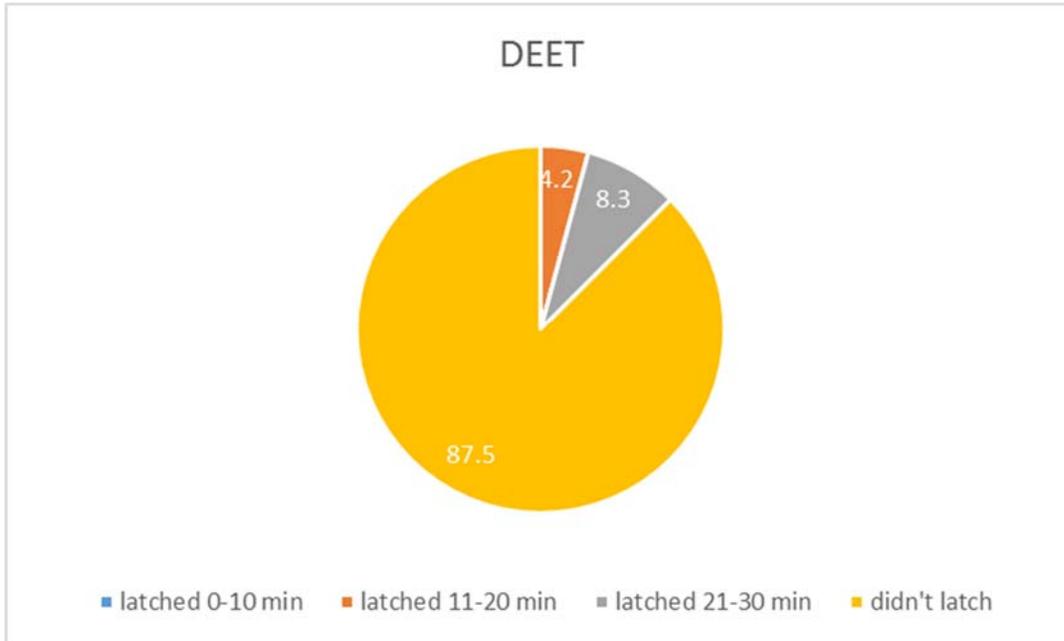


Figure 7. Scatterplot showing each treatment's starting pH against percentage of leeches that attached to the bait within 30 minutes. No significant correlation found.

